Molecular Recognition Mechanism between Mesohemin and the Antibody 11D1

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(Received November 11, 1996)

This study focuses on the molecular recognition between mesohemin and the monoclonal antibody 11D1 specific for N-hydroxymethyl mesohemin. Results showed that the antibody 11D1 bound strongly to mesohemin with a dissociate constant of 2.1×10^{-6} M. A fluorescent spectroscopic study of the mesohemin binding to the antibody 11D1 suggested the existence of an electrostatic interaction between them. The interactions among the substrate, the mesohemin and the antibody 11D1 revealed that the mesohemin inserted into the binding site of the antibody11D1 and bound to the antibody 11D1 with "edge-on" style.

Hemoproteins are known to be important functional proteins which participate in many biochemical processes, such as the transport of electrons (e.g., cytochromes), the transport of oxygen (e.g., hemoglobin), and the catalysis of redox reactions (e.g., cytochrome P450, horseradish peroxidase). Differences in the kind of protein interaction with the heme and potential substrate give rise to different functions.[1] The control mechanisms for the intrinsic reactivity of the heme are of both theoretical and practical interest. To date, much attention has been focused on molecular recognition between the heme and protein. Several groups have reported on the molecular recognition of antibodies by artificial porphyrin derivatives, in order to clarify the control mechanism for the function of hemoproteins.[2-5] To further understanding of these features of hemoproteins, we prepared the antibody 11D1 specific for N-hydroxymethyl mesoporphyrin with a partial cofactor and a substrate structure with the characteristics of horseradish peroxidase.[6]

Using the equilibrium dialysis method, the antibody 11D1 was proved to bind strongly to porphyrin compounds with the dissociation constants 1.1×10^{-6} M and 2.1×10^{-6} M for hapten and mesohemin, respectively. The binding energy difference for hapten and mesohemin was -1.46 kJ/mol. The results showed that the antibody 11D1 had similar affinity with both hapten and mesohemin. This suggests that recognition of the antibody 11D1 for porphyrin compounds occurs mainly at the porphyrin plain.

Fluorescence spectroscopy has been proposed as a unique tool for the analysis of protein structure in solution because fluorescence spectra are sensitive to variations in protein structure. Since the mesohemin cofactor has weak fluorescence, its analogues, such as metal-free porphyrins, have often been used in place of the hemin for getting structural information about the protein binding site. These substitutions have affected neither the fluorescence of the protein nor the affinity between the porphyrin and protein.[7] In this study, mesoporphyrin was chosen instead of mesohemin for fluorescence measurement. When mesoporphyrin bound with the antibody 11D1 with a molecular ratio of 2:1, the fluorescence intensity decreased significantly and the peak shifted from 666 nm to 663 nm (Figure1). This demonstrates that there is some elctrostatic interaction between the antibody and mesohemin. Some polar amino acid residues might have been present in the antibody binding site, causing fluorescence quenching of the mesoporphyrin. In addition, when the concentration of mesohemin was raised from 0.5 uM to 2.5 uM,

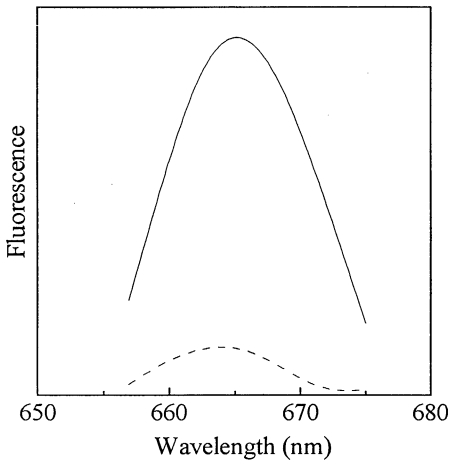

Figure 1. Fluorescence spectra of the antibody 11D1-mesoporphyrin complex (--) and mesoporphyrin (—). The excitation wavelength is 333 nm.

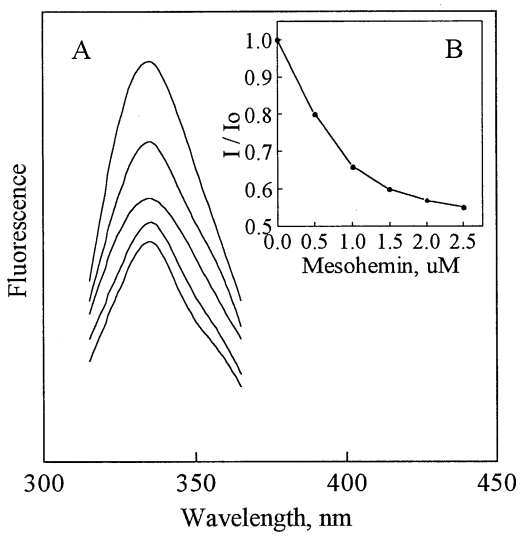

Figure 2. A. The effect of mesohemin on the fluorescence spectra of antibody 11D1. The mesohemin amounts from top to bottom are 0, 0.5, 1.0, 1.5, 2.5 uM, respectively. Antibody concentration is 0.5 uM. Excitation wavelength is 280 nm. **B.** Fluorescence quenching of antibody 11D1 by mesohemin. The fluorescence intensity was measured at 336 nm. I, fluorescence intensity of antibody 11D1 in the presence of mesohemin; Io, the initial fluoresecence intensity of antibody 11D1.

with a fixed concentration of the antibody 11D1 (0.5 uM), the fluorescence intensity of the antibody 11D1 at 336 nm gradually decreased (Figure 2A). The antibody also showed fast and slow phase fluorescence change with the increase in the amount of mesohemin(Figure 2B). The slow phase might come from the unspecific absorption of mesohemin to the fluorescence of proteins, which was verified by the interaction between the unrelated Ig G and mesohemin. The fast phase of fluorescence quench of the antibody 11D1 might be caused by the specific interaction with mesohemin. This suggests that the polar groups of mesohemin inserted into the binding site of the antibody. Two tryptophan groups were located in the binding site of the antibody, which was verified by N-bromosuccinimide chemical modification.[8] When the mesohemin combined with antibody 11D1, its carboxyl group might have entered the combining site of antibody 11D1, interacted with the tryptophan group inside, and affected the microenvironment of tryptophan located in the binding site.

The sequence of adding the antibody 11D1 (0.75 uM) and mesohemin (0.5 uM) into the substrate solution (H_2O_2 and pyrrogallol) affected the catalytic antibody activity significantly. The following two cases were taken into consideration: (1) the mesohemin was added to the substrates solution first, and then the antibody 11D1 was added; (2) the antibody 11D1 and the mesohemin were incubated first, and then the mixture was added to the substrate solution. In case (1), the rate of pyrogallol peroxidation (at 18 ℃, within 10 min) was the same as that of mesohemin (K_{cat}/K_m: 0.21 min^{-1} mM^{-1}). This may be because a large steric constraint was formed in the center of mesohemin, preventing the combination with the antibody 11D1. It also indicates that the combining site of the antibody 11D1 was not very large. Case (2) revealed that the antibody 11D1-mesohemin complex could bind easily to H_2O_2 and catalyze the peroxidation (K_{cat}/K_m: 6.62 min^{-1} mM^{-1}). A reasonable interpretation of the molecular recognition style between the antibody and mesohemin is shown schematically in Figure 3. The edge (Figure 3A), not the front (Figure 3B) of porphyrin circle faced to the outer pocket of the antibody 11D1 binding site, because the action between substrate and mesohemin did not affect the binding of mesohemin to the antibody 11D1 in the action style (B).

The results described above suggest the existence of some polar amino acid residues in the binding site of antibody 11D1 complex, which reflected the dominant role of electronic interactions. The recognition of the antibody for mesohemin

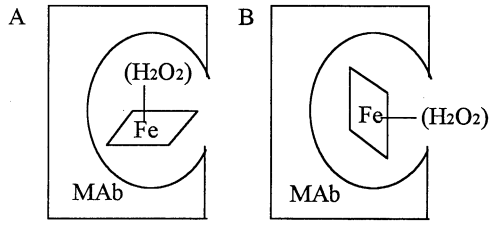

Figure 3. The proposed molecular recognition schemes for the antibody 11D1, mesohemin, and hydrogen peroxide. A: edge-on. B: side-on.

occurs mainly in the porphyrin plain; the side of porphyrin circle, not its front, faced to the outer pocket of the combining site of the antibody 11D1. This polar heme environment facilitated cleavage of the peroxide oxygen-oxygen bond; the small binding pocket and relatively inaccessible heme iron avoid oxo transfer reactions. The results of this study should promote understanding for control of the catalytic factor and be of benefit for construction of a new catalytic antibody with high hemoprotein activity.

We wish to express our gratitude to the National Education Commission Foundation of China for Ph. D training.

References

1 J. H. Dawson, *Science*, **240**, 433 (1988).
2 A. W. Schwabscher, M. I. Weinhouse, M-T. M. Auditor, and R. A. Lerner, *J. Am. Chem. Soc.*, **111**, 2344 (1989).
3 A. Harada, K. Okamoto. M. Kamachi, T. Honda, and T. Miwatani, *Chem. Lett.*, **1990,** 917.
4 A. Harada, K. Okamoto, and M. Kamachi, *Chem. Lett.*, **1991,** 953.
5 E. Keinan, S.C. Sinha, A. Sinha-Bagchi, E. Benory, M.C. Ghozi, Z. Eshhar, and B. S. Green, *Pure &Appl. Chem.*, **62,** 2013(1990).
6 Y. Feng, Z. Liu, S. J. Gao, X. Y. Liu,and T. S. Yang, *Ann. New York Acad. Sci.*, **750**, 271 (1995).
7 N. N.Ugarova, A. P. Savitski, and I. V. Berezin, *Biochim. Biophys. Acta.*, **622**, 210 (1981).
8 T. F. Spanda and B. Witkop, *Methods in Enzymology.* **11,** 498 (1967).